Drug resistance in cancer: Principles of emergence and prevention

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Although targeted therapy is yielding promising results in the treatment of specific cancers, drug resistance poses a problem. We develop a mathematical framework that can be used to study the principles underlying the emergence and prevention of resistance in cancers treated with targeted small-molecule drugs. We consider a stochastic dynamical system based on measurable parameters, such as the turnover rate of tumor cells and the rate at which resistant mutants are generated. We find that resistance arises mainly before the start of treatment and, for cancers with high turnover rates, combination therapy is less likely to yield an advantage over single-drug therapy. We apply the mathematical framework to chronic myeloid leukemia. Early-stage chronic myeloid leukemia was the first case to be treated successfully with a targeted drug, imatinib (Novartis, Basel). This drug specifically inhibits the BCR-ABL oncogene, which is required for progression. Although drug resistance prevents successful treatment at later stages of the disease, our calculations suggest that, within the model assumptions, a combination of three targeted drugs with different specificities might overcome the problem of resistance.

multiple-drug therapy | mutations | stochastic models

Drug resistance is a frequent clinical problem for cancer patients (1). Many mechanisms of drug resistance have been found (2, 3). For example, drugs can be prevented from entering the cells; drugs can be pumped out of cells; they can be enzymatically inactivated; drug activity can be prevented by mutation or altered expression of the target; and defects in apoptosis, senescence, and repair mechanisms can contribute to resistance. A particular problem in cancer is the occurrence of multidrug resistance. Many anticancer drugs cause direct damage to DNA, which triggers cellular checkpoints (4). In recent years, however, there has been a transition away from classic cytotoxic and hormonal agents toward targeted therapy (5). This involves the correction of precise molecular abnormalities that underlie the progression of the tumor. An example is the treatment of chronic myeloid leukemia (CML) with imatinib (Novartis, Basel) (6). Although such therapies have shown remarkable clinical success, the emergence of drug resistance poses problems, especially at more advanced stages of cancer (7, 8). To manage this problem, it is important to gain an understanding of the principles that underlie the emergence of drug resistance. This requires a mathematical framework. In the context of viral infections such as HIV, mathematical analysis of the evolution of drug resistance has contributed to devising the combination therapies that now successfully prevent pathology over long periods of time (9–11). This paper provides an up-to-date mathematical framework for the targeted treatment of cancer, which elucidates the principles according to which resistant tumor cells evolve. It can be applied to specific cancers that can be treated with targeted therapy. As an example, we consider CML and provide guidelines on how many drugs should be used in combination to avoid treatment failure.

The Conceptual Framework

To understand how resistant mutants are generated during cancer progression and treatment, we have developed the following conceptual framework. Cancerous cells are described by a stochastic birth–death process with a positive net proliferation rate. If we denote the growth rate of cells as L and the death rate as D, the condition $L > D$ corresponds to a clonal expansion. We further assume that cancer is detected when the colony reaches a certain size, N, at which moment therapy starts (we will also refer to N as "treatment size"). The effect of therapy is modeled by the drug-induced death rate, H, which shifts the balance of birth and death such that the colony shrinks. That is, the net cell death rate is now larger than the birth rate, $D + H > L$. If all cancerous cells were susceptible to the drug, then therapy would inevitably lead to eradication of cancer. However, in the course of cancer progression, mutations can lead to the generation of cell types that are resistant to the drug. This is assumed to occur with a probability u upon cell division. Before the tumor is treated, the mutant will behave identically compared with the wild type. During therapy, however, the resistant phenotype will proliferate, whereas the wild type will be killed with a rate H. The resulting treatment failure can be countered by combining several drugs, as demonstrated effectively with viral infections (12). Such combination therapy is included in this framework. In our first model, we assume a mutation that confers resistance to one drug does not confer resistance to any of the other drugs in use. This may not be the case for all drugs/mutations, and these effects have to be accounted for in further modifications of the model (see *Model Extension and Applicability*). With these assumptions, to become resistant to n drugs, the cell has to accumulate n mutations. These mutational processes can be presented as a combinatorial mutation network, an example of which is presented in Fig. 1. For simplicity, we assume mutant cells that are not resistant to all drugs in use are killed with the same rate as wild-type cells. Alternatively, it can be assumed that such mutants are partially resistant [i.e., are affected less than the wild-type but more than the fully resistant phenotype (13, 14)], but it turns out that this complication does not alter our results significantly (see below). The model is based on mathematical analysis of stochastic birth and death processes on combinatorial mutation networks. The approach is described in the supporting information, which is published on the PNAS web site, to keep the main text accessible for a biological readership.

We will explore the principles according to which resistant mutants are generated during the pretreatment growth phase and during therapy. In particular, we investigate the chances that resistant mutants preexist before treating a tumor of size N. In this respect, it is key to examine the number of cell divisions that occur during the growth phase until size N is reached. This is roughly given by $\mathcal{N} = NL/(L-D)$. We can see that if $D = 0$ or $D \ll L$, the number of cell divisions is approximately given by $\mathcal{N} \approx N$. On the other hand, if D is close to L ($D \approx L$), many more cell divisions are required to reach size N, because a high death

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Abbreviation: CML, chronic myeloid leukemia.

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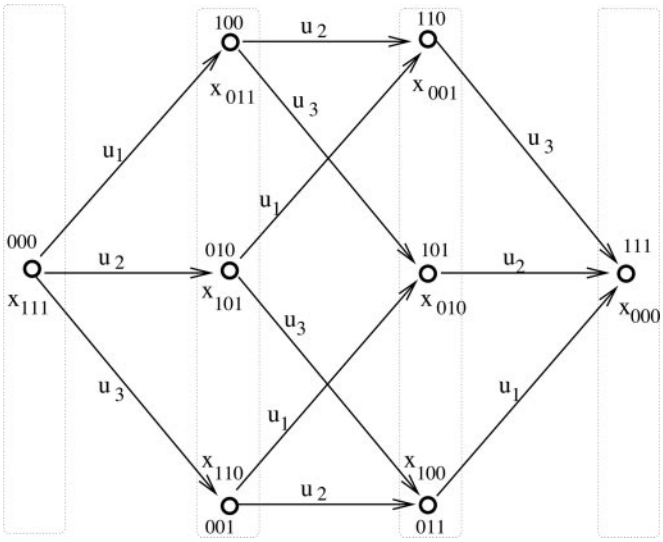

Fig. 1. Mutation diagram corresponding to three drugs. Each node corresponds to a phenotype. The binary number above each node identifies which drugs the phenotype is resistant to, e.g., 011 means this type is resistant to drugs 2 and 3 but not to drug 1. The leftmost type (000) is fully susceptible, and the rightmost one (111) is resistant to all three drugs. The mutations rates are marked above each arrow. The notation below the nodes identifies the variable used to describe each phenotype; see supporting information for details.

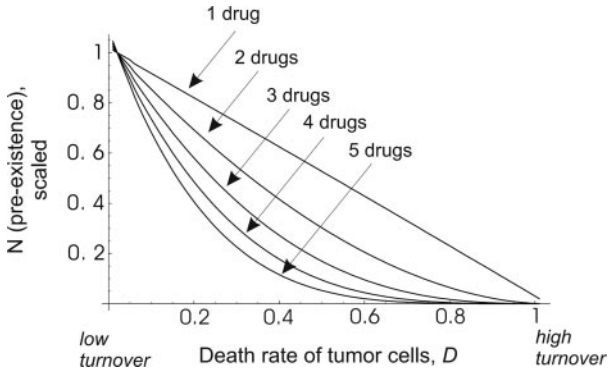

Fig. 2. Probability of producing resistant mutants before treatment, depending on the death rate of tumor cells, D. To consider the pretreatment phase only, we artificially set the mutation rate to zero upon start of therapy. Further, to concentrate on the production of resistant mutants, we assume that the mutants do not die. We plot the tumor size, N, at which the probability of treatment failure due to preexistence equals δ. Note that all curves are scaled to be displayed on one graph. For a single drug, this dependence is linear. For a larger number of drugs, this dependence becomes increasingly stronger than linear. Parameter values were chosen as follows: $L = 1$, $u = 10^{-6}$, $\delta = 0.01$.

rate cancels the effect of cell divisions. For convenience, we will call the scenario where $D \approx L$ a high-turnover cancer. In contrast, we will call the scenario where $D = 0$ or $D \ll L$ a low-turnover cancer. In the following, we will first examine the emergence of resistance against a single drug and then expand the analysis to include the use of more than one drug.

Evolution of Resistance Against a Single Drug

Consider the use of one drug only. We determine the relative roles of the pretreatment and the treatment phase for the generation of resistant mutants. In other words, how important is the preexistence of mutants? We first perform *in silico* experiments, where we artificially set the mutation rate to zero right after treatment starts. That is, mutations can be generated only before therapy. We calculate the probability of treatment failure in this setting, which we define as the probability that the cancer escapes therapy due to generation of resistant mutants. This is denoted by $P_1^{\uparrow}$; the symbol $\uparrow$ indicates that we look at the contribution of the growth phase to mutant generation, and the subscript 1 refers to a one-drug therapy. Next, we set the mutation rate to zero in the pretreatment phase. Now, mutations can be generated only during therapy, and we can evaluate the corresponding contribution to treatment failure, $P_1^{\downarrow}$. It turns out that for realistic treatment regimes, we have $P_1^{\uparrow} > P_1^{\downarrow}$. That is, the generation of resistant mutants takes place predominantly before treatment starts. The treatment phase becomes important only for the generation of resistance in the unrealistic case where $H \leq H_c$ [where $H_c \equiv 2(L-D)$]. Under this condition, treatment is very ineffective, such that the number of cell divisions during treatment is higher than during the growth phase before treatment. In other words, the time it takes to eradicate the tumor by drugs in the absence of resistance is larger than the age of the tumor upon start of therapy! This is not a likely scenario. Therefore, we conclude that for all realistic cases, resistance develops before the start of treatment.

Combining the growth and treatment phases, we can calculate the overall probability of treatment failure as a function of treatment size, N. We have

$$P_1^{\text{tot}} \approx 1 - \left(1 - \frac{Hu}{H + D - L}\right)^N.$$

Importantly, if H is large relative to D, then

$$P_1^{\text{tot}} = P_1^{\uparrow} = 1 - e^{-Nu}, \qquad [1]$$

and it is independent of the turnover rate. This result means that in the context of a single drug, high- and low-turnover cancers behave in exactly the same way, so far as the preexistence of mutants is concerned. An intuitive explanation is as follows. A higher-turnover cancer requires more cell divisions to reach size N, and thus more mutants are created. At the same time, however, the death rate of the mutants is also increased. The two effects cancel each other out. Similar behavior was observed numerically in ref. 15. The contribution of the treatment phase can be ignored, and the occurrence of treatment failure is not influenced by the efficacy of the drugs.

Evolution of Resistance Against Two or More Drugs

Now, consider treatment with two or more drugs. We observe an important difference compared with the one-drug scenario above: The probability that resistant mutants preexist now depends on the natural death rate, D (i.e., the dynamics are different for high- and low-turnover cancers; see Eq. **1**). The larger the number of drugs in use, the stronger this dependency (Fig. 2). The key to understanding this lies in the process of mutant generation. To explain this, assume that once produced, a mutant does not die. In the context of one drug, the probability that at least one resistant mutant has been produced in the course of tumor growth up to size N is given by $u\mathcal{N} = NLu/(L-D)$. This depends linearly on $(L-D)^{-1}$ (Fig. 2), and is canceled out by the factor $(1-D/L)$ if mutant death is included. For two drugs (requiring a double mutant), this probability is roughly given by

$$2\left(\frac{Lu}{L - D}\right)^2 N \log N.$$

The dependence on $(L-D)^{-1}$ is now stronger than linear and is not canceled out anymore if mutant death is included. In general, if the number of drugs is increased, a higher natural death rate

Table 1. The $\log_{10}$ size at which resistance becomes a problem (i.e., treatment failure in >1% of patients), depending on the number of drugs and the rate at which resistant mutants are generated, *u*

	No. drugs					
u	One	Two	Three	Four	Five	Six
10^{-4}	2.01	4.95	7.46	9.81	12.06	14.23
10^{-5}	3.01	6.73	10.13	13.36	16.70	20.02
10^{-6}	4.01	8.61	12.91	17.04	21.49	25.83
10^{-7}	5.01	10.53	15.75	20.8	26.17	31.43
10^{-8}	6.01	12.47	18.62	24.6	30.90	37.10
10^{-9}	7.01	14.42	21.36	28.23	35.61	42.86

If we assume that the cancers cannot grow beyond 10^{13} cells without causing death, a treatment regime can be considered acceptable if resistance becomes a problem only at sizes $>10^{13}$ cells (i.e., $\log_{10}$ of the size >13). The parameter regimes where this occurs and where treatment is expected to be successful are indicated by shading. Calculations assume $L = 1$, $D = 0$.

of tumor cells, D, contributes increasingly to the production of resistant mutants and thus to treatment failure (Fig. 2). Another difference compared with the one-drug scenario is that with higher numbers of drugs, the treatment phase becomes completely insignificant. For instance, for $n = 2$ drugs and $D = 0$, we have

$$P_2^{\uparrow} = 1 - (1 - 2\log Nu^2)^N > P_2^{\downarrow} = 1 - (1 - 2u^2)^N.$$

The reason lies in the dynamics of the intermediate mutants. During the growth phase, a cell with a single mutation will undergo clonal expansion, and this facilitates the generation of further mutations. During the treatment phase, a cell with a single mutation has a negative growth rate (because it is susceptible to one or more drugs), and this makes it unlikely that additional mutations can be attained before the clone is extinct.

Summary of the Evolutionary Dynamics of Resistance

All of the above arguments (and calculations) can be summarized as follows. For the case of one drug, the probability of treatment failure for a given size is independent of whether the cancer has a high- or a low-turnover rate. The contribution of the pretreatment phase to the generation of resistance is greater than that of the treatment phase as long as $H > H_c$. On the other hand, for two or more drugs, we have:

- Pretreatment phase always plays the dominant role in treatment failure, and generation of resistance during treatment can be ignored, $P_n^{\uparrow} \gg P_n^{\downarrow}$ for $n > 1$;
- High-turnover cancers have a higher probability of treatment failure (for the same size N) than low-turnover cancers;
- Both of these effects become stronger for larger numbers of drugs.

Prevention of Resistance

After examining the basic evolutionary dynamics of drug resistance in cancer, we turn to a more applied question: How many drugs should be used to prevent treatment failure depending on the size of the tumor? We address the problem of treatment failure in the following way. We ask at which tumor size N the probability of treatment failure reaches a threshold value, which we denote by δ. This means that if we start treatment at tumor size N, failure will be observed in a fraction δ of the patients, whereas treatment will be successful in a fraction $1 - \delta$ of patients. For now, we assume that an acceptable goal is to treat 99% of patients successfully, that is $\delta = 0.01$. Table 1 shows the tumor sizes at which resistance becomes a problem (i.e., <99% of patients will be treated successfully), depending on the rate at which resistance mutations are generated, u, and the number of drugs, n.

Assume that a single drug is used to treat patients. The tumor size when resistance arises is given by

$$\log N = \log\left[\frac{\delta(H + D - L)}{H}\right] - \log u.$$

Resistance arises at lower tumor sizes for higher mutation rates, u (Fig. 3*a*). Note that if $H \gg D$, we have a very simple relation, $\log n = \log \delta - \log u$. That is, the results are not influenced by the natural death rate, D. This is in accordance with our theoretical reasoning above.

If the number of drugs is increased, we observe three important differences:

1. An increase in the mutation rate, u, results in a more pronounced decline of the tumor size when resistance is observed. The larger the number of drugs, the more pronounced this decline (Fig. 3*a*).
2. The treatment phase, and thus the treatment efficacy, H, has no influence on the generation of resistance.
3. The size at which resistance arises now depends on the death rate, D. Resistance arises at smaller tumor sizes if the death rate of tumor cells, D, is higher (Fig. 3*b*). The larger the number of drugs and the higher the mutation rate, u, the stronger this dependency (Fig. 3*b*).

By how much does an increase in the number of drugs improve the chances of treatment success? According to the arguments above, this depends on the mutation rate, u, and the death rate of tumor cells, D. (*i*) The higher the rate at which resistance mutations are acquired, u, the less is the effect of adding another drug, and the more difficult it becomes to treat (Fig. 3*c*). Consider the most optimistic scenario where $D = 0$ (Table 1). Assuming that cancers can reach up to sizes of 10^{13} cells (16), $u = 10^{-9}$ requires two drugs, $u = 10^{-7}$–10^{-8} requires three drugs, $u = 10^{-5}$–10^{-6} requires four drugs, and $u = 10^{-4}$ requires six drugs (Table 1). By extrapolation, 10 drugs are needed if $u = 10^{-3}$, and ≈30 drugs if $u = 10^{-2}$. Therefore, drugs to which resistance can be generated with such high rates (e.g., because genetic instability happens to promote the necessary mutations) should not be developed. (*ii*) As pointed out above, resistance arises at lower tumor sizes as the death rate, D, is increased. In fact, if the death rate of tumor cells, D, comes close to their division rate, L (high-turnover cancer), then the effect of combining multiple drugs disappears (Fig. 3*b*). The size at which resistance arises converges to the same value, no matter how many drugs are used. In this case, the frequency with which

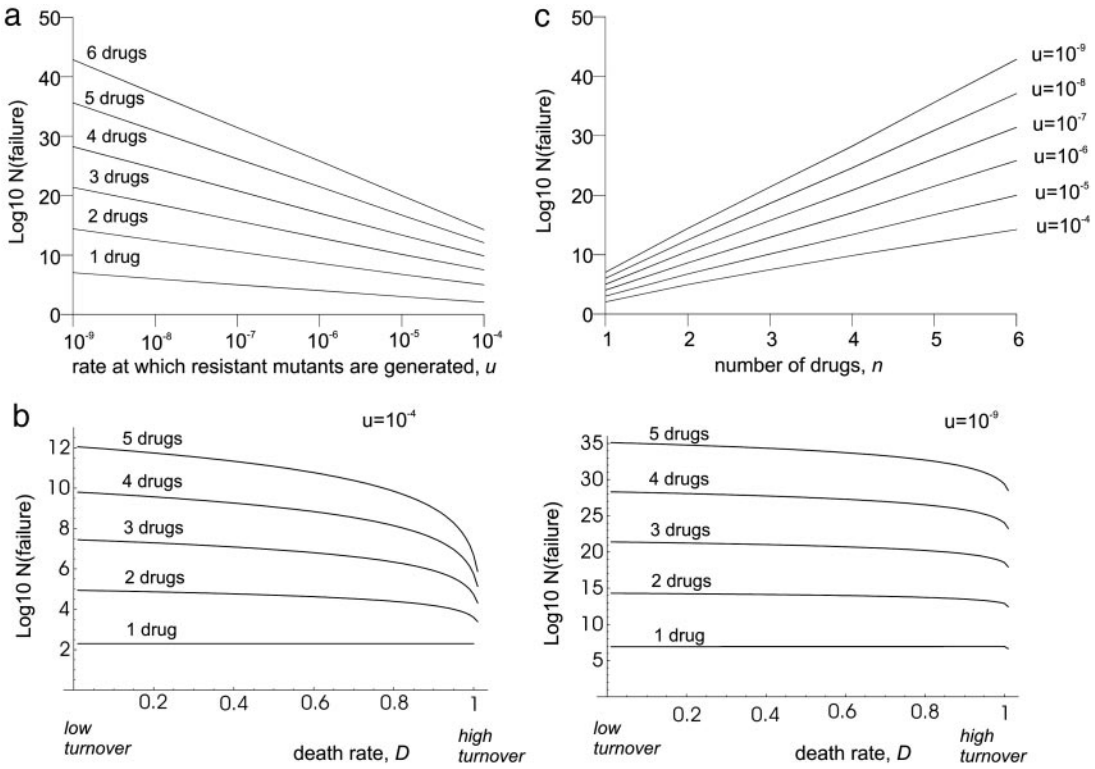

Fig. 3. Log tumor size, *N*, at which treatment failure is observed, depending on the parameters of the model. (*a*) Dependence on the rate at which resistant mutants are generated, *u*. The higher the value of *u*, the lower the tumor size at which treatment fails. The larger the number of drugs, the stronger this dependency. (*b*) Dependence on the natural death rate of tumor cells, *D*. The higher the value of *D* (i.e., the higher the turnover of the cancer), the lower the tumor size at which treatment fails. The higher the number of drugs and the rate at which resistant mutants are generated, *u*, the more pronounced this trend. (*c*) Dependence on the number of drugs, *n*. Increasing the number of drugs increases the tumor size at which treatment fails. The higher the mutation rate, however, the lower the advantage gained from adding further drugs. Baseline parameter values were chosen as follows: $L = 1$, $\delta = 0.01$.

cancers arise is low, because they have a high chance to go extinct spontaneously, but when they do arise, the chances of complete tumor eradication are very slim. Because high-turnover cancers are likely to grow relatively slowly, however, drug therapy could still increase the lifespan of the patient significantly by reducing the number of cells below a threshold rather than achieving a full response. Regrowth of resistant cells to large sizes would take a long time.

Application to CML

As a specific example, consider the treatment of CML (17), which develops in three phases: (*i*) the chronic phase, characterized by expansion of terminally differentiated cells; (*ii*) the accelerated phase, associated with a higher fraction of undifferentiated cells; and (*iii*) blast crisis, where undifferentiated cancer cells undergo large expansion in the presence of genomic instability. The initiation and further progression of CML are driven by chromosome translocation, resulting in the BCR-ABL fusion gene, which encodes a cytoplasmic protein with constitutive tyrosine kinase activity (18). The drug imatinib mesylate (Gleevec, formerly STI571; Novartis, Basel) is a small-molecule inhibitor of the Bcr-Abl kinase and can achieve sustained hematologic and cytogenetic responses in chronic phase disease. Treatment of blast crisis, however, often fails because of drug resistance (19). In accordance with our framework, it has been reported that mutants might preexist the initiation of treatment rather than being generated during the treatment phase (20, 21). Data suggest that two main types of mutations confer resistance to the cells (19, 20, 22): the amplification of BCR-ABL, or a point mutation in the target protein. Genetic instability (23) is likely to promote the occurrence of gene amplifications, which have been measured to occur in cancer cells at a rate of 10^{-4} per cell division (24). On the other hand, the point mutation rate is $\approx 10^{-9}$ per base per cell division (25). However, the frequency of gene amplifications is much less than that of point mutations among patients (20). Part of the reason might be that BCR-ABL amplifications are costly to the cells in the absence of treatment (26). Including this assumption into the modeling framework, however, shows that even if this fitness cost is very significant, amplifications should still be observed more often than point mutations (not shown). However, it is thought that the level of resistance is a function of the number of extra copies of the BCR-ABL gene. Therefore, if a significant degree of resistance requires two or more amplification events (but only one point mutation event), we expect that a resistant mutant is generated faster by point mutation than by gene amplification, explaining the observed frequencies.

Thus, for prevention of drug resistance, we assume that resistant mutants are generated maximally with a point mutation rate of $u = 10^{-8}-10^{-9}$. Experiments with susceptible CML cell lines have shown viability measurements (in the absence of treatment) of $\approx 90\%$ (26). From this, we can roughly calculate that the relative death rate of cancer cells is in the range of $D/L = 0.1-0.5$. In this parameter region, we find that a combination of three drugs should prevent resistance and ensure successful therapy even for advanced cancers (Table 2). This assumes that the size of advanced cancers is $<10^{13}$ cells, which derives from white blood cell count measurements that range from 10^5 to 10^6 per microliter of blood in blast crisis. Recent findings (27) indicate that BCR-ABL might increase the amount of reactive oxygen species and thus the rate of point mutations.

Table 2. Application to the treatment of CML blast crisis with imatinib

	No. drugs				
D/L	One	Two	Three	Four	Five
Assuming $u = 10^{-8}$					
0.1	5.95	12.34	18.45	24.38	30.19
0.5	5.95	12.13	17.99	23.69	29.26
0.9	5.95	11.48	16.70	21.74	26.66
Assuming $u = 10^{-6}$					
0.1	4.00	8.55	12.80	16.89	20.86
0.5	4.00	8.31	12.37	16.20	19.93
0.9	4.00	7.68	11.07	14.40	17.40

We give the $\log_{10}$ size at which resistance becomes a problem, depending on the number of drugs and the turnover rate of the cancer cells (value of D/L). From published data, we estimated that the value of D/L must lie between 0.1 and 0.5, and we also present calculations for $D/L = 0.9$. We consider treatment robust if resistance arises only at tumor sizes $>10^{13}$ cells (i.e., the value 13). In this case, the combination of three drugs is expected to result in the prevention of resistance and successful treatment. We consider two cases. First, we assume that resistant mutants are generated with a rate of $u = 10^{-8}$. The reason for this parameter choice is as follows: while the point mutation rate is around $u = 10^{-9}$, several point mutations can lead to resistance and this increases the rate. In the second calculation, we assume that resistant mutants are generated with an elevated rate of $u = 10^{-6}$, i.e. a 100-fold increase. This represents the borderline where three drugs will no longer prevent resistance. Thus, so long as the point mutation rate is elevated $<$100-fold by BCR-ABL, triple drug therapy should prevent resistance.

So long as the elevation of the mutation rate is $<$100-fold, our results remain robust (Table 2).

Model Extension and Applicability

In addition to imatinib, possible candidates for additional drugs to be used in combination in CML therapy have been discussed in the literature (28), although the most promising ones show some degree of crossresistance with imatinib (29). If this is the case, our framework still applies, but the calculations would have to be modified in the following way. Suppose drug X possesses crossresistance with imatinib. This means that a part (or all) of the mutants resistant to imatinib will also be (partially) resistant to drug X. In the case where they are fully resistant to both drugs, treating with the two drugs will not be more effective than treating with just one of the drugs, and the clinical strategy will have to be developed by using other important considerations such as toxicity, etc. However, if resistance to drug X is partial, then the resistant mutants will have a slower growth rate under a two-drug therapy compared with that under a single-drug therapy. In this case, we can calculate the advantage of a two-drug therapy, for instance in terms of a reduction of the tumor load. The occurrence of crossresistance is discussed in refs. 30–32.

Another important issue is the heterogeneity of tumors. In CML [as well as acute myeloid leukemia and several solid tumors including breast and central nervous system tumors (33–35)], there is evidence for the existence of cancer stem cells, comprising a fraction of the total tumor burden. For CML, the fraction of stem cells in blast crisis is $>30\%$, and it is much smaller in the chronic phase (33, 34). It has been proposed that these cancer stem cells, which are the only tumor cells that have potential for self renewal, may account for drug resistance after initial response to therapy. This circumstance can be taken into account by using the present framework. Because resistance is mainly a problem in blast crisis and usually does not arise in the chronic phase, we performed our calculations for the latter phase of the disease. During this phase, the blasts undergo a phase of rapid exponential growth, and therefore the quantitative results of our present calculations apply. However, it would be an interesting extension to consider heterogeneous populations of the chronic and accelerated phases of CML. There, stem cells constitute a smaller fraction of the total population, and the predominant division pattern is asymmetric, so one would have to make two modifications in the model: (*i*) the total (effective) population of dividing cells is smaller, and (*ii*) resistant mutants may appear by two mechanisms: as a result of a mutation upon a symmetric division of a stem cell and of an asymmetric division. It can be checked that this will lead to a lower chance of the generation of resistance compared with the blast crisis.

In the present calculations, we assumed that resistant mutants behave in the same way as the wild-type tumor cells before treatment starts. This may not be the case. If one can establish that resistant mutants possess a fitness advantage in the absence of treatment, this will definitely make the estimate of the probability of resistance generation higher. Indeed, resistant mutants will grow faster and reach higher numbers (and a larger fraction of the total tumor load) before the treatment starts. On the other hand, if resistant mutants are at a disadvantage before the beginning of therapy, this would make generation of resistance less likely. This information (as it becomes available) can be very naturally incorporated in the model by including a different growth rate (L) and death rate (D) of the mutants compared with wild type.

The subject of drug resistance in cancer is very broad, and many different scenarios are possible. For example, a targeted drug may not be directly killing cancerous cells but instead, it can selectively affect tumor-derived endothelial cells. This is the basic principle of antiangiogenic therapies (36). There, a different mechanism of resistance is possible, when hypoxia (which is a consequence of the therapy) selects for vessel-independent (and therefore resistant) cancer cells. In this case, it has been noted (37) that stronger therapies will be more effective at selecting for resistance and moreover, mutations conferring resistance can also give rise to apoptosis-resistant and increasingly malignant tumor cells. A mathematical treatment of this case is possible and will be presented elsewhere.

The point of the CML calculations presented here is to illustrate how the mathematical framework can be applied to the targeted treatment of a specific cancer, based on experimental observations. Although improved predictions will require that a higher degree of complexity is included in the model, as discussed above, the basic framework presented here can accommodate this easily. In addition, it will be interesting to take into account the many new and controversial concepts that are being discovered and discussed in the literature. The strength of our framework is that it can be used to study many complex scenarios. New information can be incorporated as it becomes available from experiments and clinical trials.

Conclusion

This paper has provided an up-to-date mathematical framework that helps us to understand the principles that underlie the emergence of resistance in cancers treated with targeted drugs. It suggests treatment schedules that maximize the chances of successful therapy. Although the model correlated treatment success with eradication of the cancer, results are unlikely to change significantly if we assume that a certain small number of cancer cells persist in a resting or dormant fashion. We have shown how the framework can be applied to a specific cancer to make predictions. CML is an obvious application, because blast crisis corresponds to the clonal expansion processes described by our model, and drug activity, as well as resistance mechanisms, is well defined. The mathematical framework can be adapted to take account of more complicated cancer growth patterns. We

have shown how nonneutral resistant mutants, stem-cell/differentiated cell hierarchy, and the effect of partial crossresistance can be incorporated into the model, as the data become available.

Of particular importance for the basic model is the measurement of cellular turnover kinetics at different stages of the disease. The rate of cell division, L, and of cell death, D, can be calculated from DNA-labeling data, similar to studies performed in the context of immunology (38). It will also be interesting to explicitly take into account the kinetics of cancer decay during treatment, to address further details, such as the occurrence of primary resistance (no response after initial treatment) vs. secondary resistance (relapse after an initial response).

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